## REMARKS

This is in response to the Office Action that was mailed on December 13, 2002. Applicants gratefully acknowledge the indicated allowability of claims 15-17. Claims 3-14 are cancelled -- without prejudice to their reassertion in this or a continuing application -- in order to expedite prosecution. Claim 1 is partially rewritten to clarify the relationship between the mRNA recited in the claim and the nucleic acid which is the subject of the claim. Typographical errors are corrected throughout the specification. No new matter is introduced by this Amendment. Entry of the present Amendment in order to place the application into condition for allowance, or into better condition for appeal, is respectfully solicited. With this Amendment, claims 1 and 15-18 are in the application.

WRITTEN DESCRIPTION

Claim 1 was rejected under the first paragraph of 35 U.S.C. §112 as being drawn to an invention that is not described in the manner prescribed by the statute. This ground of rejection is respectfully traversed.

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species *OR* by disclosure or relevant identifying characteristics, such as structure or other physical and/or chemical properties, *OR* by functional characteristics coupled with a known or disclosed correlation between function and structure. MPEP 2163, II.A.3.a.ii. See *University of California v. Eli Lilly*, 43 USPQ2d 1398 at 1406. Claim 1 states that the sequences which are claimed in addition to SEQ ID NO:1 and SEQ ID NO:3 must (i) have the defined degree of similarity to those specified sequences, AND (ii) be hybridizable to those sequences under high stringency conditions, AND (iii) must meet the recited functional criterion.

The present specification provides detailed and extensive disclosure relating to the "similarity" recited in the claim, starting in line 6 on page 22 of the specification and continuing through line 8 on page 26 of the specification. "Where there is non-identity at the nucleotide level, 'similarity' includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels." "... sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a 'comparison window' to identify and compare local regions of sequence similarity." "For the purposes of the present invention, 'sequence identity' will be understood to mean the 'match percentage' calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software Engineering Co., Ltd., South San Francisco, California, USA) ...." "... the present invention contemplates a method for the construction of a nucleic acid molecule comprising a non-naturally occurring nucleotide sequence, said method comprising constructing in a particular reading frame, a contiguous sequence of codons which encode a sequence of amino acids of a polypeptide where one or more codons are selected to express at a higher level in a particular host cell or *in vitro* expression system relative to the corresponding codons in the naturally occurring nucleotide sequence encoding the same polypeptide, wherein the selected codons are preferably used by a host cell, and wherein the codon for Phe may be selected from the group comprising UUU and UUC, the codon for Ser may be selected from the group comprising UCU, UCC, UCA, UCG, AGU and AGC, the codon for Tyr may be selected from the group comprising UAU and UAC, ... the codon for Glu may be selected from the group comprising GAA and GAG, and the codon for Gly may be selected from the group comprising GGU, GGC, GGA, and GGG." The

specification herein make it abundantly clear that Applicants were in possession of the invention recited in claim 1.

The PTO has provided guidelines for applying the written description requirement to biotechnology applications. Example 9 in those guidelines deals with hybridization. In guideline Example 9, the claim in question is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO:1 and must encode a protein with a specific activity. Highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) are disclosed. The application in the guidelines discloses only one species (SEQ ID NO:1 itself) falling within the scope of the claimed genus. The analysis provided by the guidelines is as follows:

> ... a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Similarly, in the present application, the claim in question is drawn to a genus of nucleic acids all of which must hybridize under stringent conditions with a specified sequence and all of which have a recited useful property (expressing differential amounts of mRNA in a normal vs. diseased state of a subject, which difference is detectable.). The nucleic acids claimed here are even further described as -- in addition to the two factors paralleling those in the guidelines -- having at least about 60% similarity to the full length of the specified sequences.

Manifestly, claim 1 is in fact drawn to an invention described in the manner prescribed by the first paragraph of 35 U.S.C. §112 .

SCOPE

Claim 1 was rejected under the first paragraph of 35 U.S.C. §112 as being drawn to an invention which is claimed in a manner that exceeds the scope of the enablement. The rejection is respectfully traversed.

The Examiner apparently agrees that those skilled in the art would have no difficulty in determining whether a given nucleotide sequence has at least about 60% similarity to SEQ ID NO:1 or to SEQ ID NO:3 after optimal alignment, and in then determining whether that similar sequence is capable of hybridizing to SEQ ID NO:1 or to SEQ ID NO:3 under high stringency conditions defined as 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C. The Examiner disagrees, however, that a person skilled in the art could then determine by routine screening whether mRNA corresponding to that sequence is differentially or preferentially expressed in human hepatocellular carcinoma tissue or tissue from pancreatic adenocarcinoma relative to other tissue in said subject and/or in subjects not diagnosed with this condition.

As discussed in the present specification, some genes are expressed preferentially or exclusively during particular disease conditions such as cancer or autoimmune conditions. The identification of such genes provides a basis for, for instance, diagnosis and developing protocols for down-regulating expression of the gene. Paragraph [0010] herein teaches that a marked increase in hcc-1 cDNA level is observed in pancreatic adenocarcinoma, and that an increase in hcc-1 cDNA level is also observed in well-differentiated hepatocellular carcinoma. Paragraph [0024] teaches that reference herein to an "expression product" includes reference to mRNA transcribed from a nucleotide sequence of a gene and/or an amino acid sequence. These expression products may be identified directly, or they may be identified

indirectly, for instance via a complex (e.g., tRNA-amino acid complex) or via an effect.

The Examiner argues that

> In order to determine which other SEQ ID NO:1 or 3-related nucleic acid sequences are biomarker nucleic acid molecules for pancreatic adenocarcinoma or heptocellu[l]ar carcinoma, one [skilled in the art] has to identify which other nucleic acid molecules are differentially expressed in those cancer patients ....

In determining whether an isolated nucleic acid falls within the scope of claim 1, a person skilled in the art would first determine whether the nucleic acid in question had at least about 60% similarity to SEQ ID NO:1 or to SEQ ID NO:3. Assuming the nucleic acid in question passed that test, the person skilled in the art would then determine whether it hybridizes to SEQ ID NO:1 or to SEQ ID NO:3 under the stringent test recited in claim 1. If the nucleic acid in question were found to pass this second test too, it would necessarily have a great deal in common structurally with SEQ ID NO:1 or SEQ ID NO:3, and its expression profile would likely parallel those of the reference sequence. With all of these limitations and sources of guidance built in by claim 1, it is not seen that an unduly large number of clinical and control samples, as argued by the Examiner, would be required to fine tune the analysis of any differences between the relevant expression profile of the candidate nucleic acid and the expression profile of SEQ ID NO:1 or SEQ ID NO:3. Moreover, the specification herein -- see e.g. paragraphs [0114] and [0115] -- provides ample exemplification of how to determine whether a nucleic acid meets the requirements set forth in claim 1.

It is respectfully submitted that the genus defined by the present claims is clearly delimited, fully supported, and does not require undue

experimentation, given the explanatory disclosure in the specification and the sophistication of those skilled in the relevant art.

PRIOR ART

Claim 1 was rejected under 35 U.S.C. §102(b) as being anticipated by WO 9845436 A2 (Agostino). The Examiner alleges that

> ... the claim is not limited to 60% similarity to the entire SEQ ID NO:1. The claim could be also interpreted as drawn to any nucleotide sequence that hybridizes to SEQ ID NO:1 under the conditions specified in the instant claim.

Office Action, paragraph bridging pages 3-4. In other words, the Examiner is construing the claim as requiring only similarity or hybridization. However, the claim expressly requires "a nucleotide sequence, ***having*** at least about 60% ***similarity to the full length*** of SEQ ID NO:1 or SEQ ID NO:3, ***that hybridizes*** to SEQ ID NO:1 or SEQ ID NO:3". Emphasis supplied. Thus claim 1 requires that the sequences in question both have similarity to the full length of the specified sequences AND hybridize thereto. The rejection as stated by the Examiner is untenable, and should be withdrawn.

SUPPORT

Claim 18 was rejected under the first paragraph of 35 U.S.C. §112 as being drawn to an invention that is not adequately supported in the specification. The rejection is respectfully traversed.

The specification as filed contained a description of each and every nucleotide in SEQ ID NO:1. Claim 18 simply recites some but not all of those expressly disclosed nucleotides. Therefore there is no basis for the contention that the specification does not convey that Applicants had possession of the expressly disclosed nucleotides contemplated by claim 18. It is noted, also,

that paragraph [0080] herein addresses the concept of "only a portion of the complete polynucleotide sequence".

**Conclusion**

It is respectfully requested that this application be passed to issue with claims 1 and 15-18. Applicants reserve the right to file divisional applications covering the inventions of claims 3-14.

If the Examiner has any questions concerning this application, he is requested to contact Richard Gallagher, Reg. No. 28,781, at (703) 205-8000 in the Washington, D.C. area.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,
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By _____

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Attachment: Version with Markings to Show Changes Made

***Version with Markings to Show Changes Made:***

In the Specification:

Paragraphs [0008], [0009], [0027], [0064], [0102], [0105], [0106], and EXAMPLE 16 [0126]-[0129] have been amended as follows:

[0008] A novel protein, HCC-1, is identified from the HCC-M cell line through a 2D gel electrophoresis and mass spectrometry analysis of the cell proteome. The assembled EST sequence of the novel protein is confirmed by a peptide mass fingerprinting and RACE. The coding region of **[**Hcc-1**]** hcc-1 cDNA has 630 bases, which code for the 210 amino acids of the full-length protein. The unique DNA sequence at the 3' untranslated region (218 bp) has been used to localize the gene to chromosome 7q22.1. A total of 690 bp at the 5' untranslated region of hcc-1 **[**Hcc-1**]** has been identified and promoter activity has been demonstrated at this region. A number of uORFs, which is a common feature in proto-oncogenes and growth factors, are noted at the 5' untranslated region.

[0009] The protein HCC-1 is localized to the nucleus region of two liver cell lines by immunofluorescence staining. Bioinformatics predictions show that the first 42 amino acids of the protein have identity matches to heterogenous nuclear ribonucleoproteins from various vertebrate species including human. The domain is also a putative bi-helical DNA-binding motif. The rest of the **[**hcc-1**]** HCC-1 amino acid sequence has no known homology in vertebrates.

[0027] The term "differential" or a related term such as "differentially" in relation to gene expression means that a gene sequence is expressed in one type of cell or tissue (e.g. cancerous cell or tissue) but is substantially not expressed in another cell or tissue. The term "preferential" or a related term such as "preferentially" **[**□preferentially□**]** in relation to gene expression means that a gene sequence is expressed at a higher level in one type of cell or tissue (e.g. cancerous cell or tissue) relative to another type of cell or tissue. The difference in expression levels may, for example, be from two-fold to 100-fold or from three-fold to 50-fold. In one embodiment, the gene is liver tissue of patients within hepatocellular carcinoma and is substantially not expressed in the normal liver.

[0064] The binding processes are well-known in the art and generally consist of cross-linking, covalently binding, or physically adsorbing. The [, the] polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to 25°C [25□C] or above) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

[0102] The first dimensional IEF was performed on precast 18 cm IPG strips (Amersham Pharmacia Biotech) at 20°C with a maximum current setting of 50 μA/strip using an Amersham Pharmacia IPGphor IEF unit. The strips were rehydrated for a minimum of 10 hrs in ceramic strip holders in 350 EL [□L] of sample containing 7 M urea, 2 M thiourea, 4% v/v CHAPS, 1 mM PMSF, 20 mM dithiothreitol (DTT) (Bio-Rad) and 0.5% v/v IPG buffer (Amersham Pharmacia Biotech). The amount of protein loaded was ~150 μg for analytical gels and ~400 μg protein for preparative gels. A low voltage of 30 V was applied during rehydration. After rehydration, IEF run was carried out using the following conditions: (i) 500 V, 500 Vhr; (ii) 1,000 V, 1000 Vhr; and (iii) 8000 V, 32000 Vhr. Voltage increases were performed on a step-wise basis. Before carrying out the second-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the strips were subjected to a two-step equilibration. The first was an equilibration buffer consisting of 6 M urea, 30% v/v glycerol (BDH Laboratory Supplies, Poole, England), 2% w/v SDS (Merck KGaA, Darmstadt, Germany), 50 mM Tris-HCl (pH 6.8) and 1% w/v DTT. The second step was with a buffer consisting of 6 M urea, 30% v/v glycerol, 2% w/v SDS, 50 mM Tris-HCl (pH 8.8) and 2.5% w/v iodoacetamide (IAA) (Sigma). After the IPG strips were transferred onto the second-dimension SDS-PAGE gel, the strips were sealed in place with 0.75% agarose (USB). SDS-PAGE was performed on 1.0 mm thick 10% and 10% w/v polyacrylamide gels at a constant voltage of 110 V at 10°C using an Amersham Pharmacia Iso-Dalt electrophoresis unit.

[0105] Silver stained spots were excised manually with a homemade plastic plunger and transferred to a 96-well polypropylene [microtitre] microtiter plate. Each excised spot [spots] was washed with 175 μL [ΦL] of 25 mM Tris-HCl (pH 8.5) in 50% acetonitrile (Applied Biosystems, Foster City, CA, USA). The plate

was sealed with an adhesive film and stored at 4°C for at least 24 hrs. This step was critical for the equilibration of gel spots as it allowed for more efficient enzyme digestion. Prior to the addition of trypsin, the washing solution was replaced with a fresh aliquot of solution and plates were incubated with shaking for 20 mins at 37°C. The washing solution was then aspirated and gel spots were dried in a Savant Automatic Environment SpeedVac AES2010 centrifugal concentrator (Holbrook, NY, USA) for 30 mins. Enzymatic digestion was performed with the addition of 10 µL [ΦL] of 0.02 µg/[°]L trypsin (Promega Corporation, Madison, WI, USA) in 25 mM ammonium bicarbonate (pH 8.5) (Sigma) to each gel piece and incubated at 37°C [37□C] overnight with shaking. To enhance peptide extraction, 10 ΦL of 0.1% trifluoroacetic acid (TFA) (Sigma) in 50% acetonitrile was added to each well and the [microtitre] microtiter plate sonicated for 10 mins in an ultrasonic water bath (Crest Ultrasonics, NJ, USA).

[0106] Mass analyses were performed according to [a] previously published methods using a PerSeptive Biosystems Voyager-DE STR MALDI-TOF MS (Framingham, MA, USA). In essence, 1 µL [ΦL] of the extracted sample from each of the microtitre wells was dispensed onto a MALDI sample plate along with 1 µL [□L] of matrix solution (10 mg/mL α-cyano-4-hydroxycinnamic acid (Sigma), 0.1% TFA, 50% acetonitrile). The samples were allowed to dry under ambient conditions. For each sample, the average of 256 spectra was acquired in the delayed extraction and reflector mode. The average of 4 scans (each containing 64 spectra) that passed the accepted criterion of peak intensity was automatically selected and saved. Spectra were automatically calibrated upon acquisition using a two-point calibration with residual porcine trypsin autolytic fragments (842.51 and 2210.10 $[M+H^+]$ ions). Assignment of peaks was done manually, measured peptide masses were excluded if their masses corresponded to trypsin autodigestion products or [from] to identified proteins adjacent to the spot being analyzed.

EXAMPLE 16 - Bioinformatics Findings on HCC-1 [Hcc-1]

[0126] The Conserved Domain Database (CDD) with Reverse Position Specific BLAST search on the 1-42 amino acids of HCC-1 [Hcc-1] gave the result as a SAP domain (e-value of 5e-04), which is a putative bi-helical DNA-binding motif predicted to be involved in chromosomal organization and transcriptional regulations (Massari & Murre 2000) found in diverse nuclear proteins. This is supported by PredictProtein where amino acid sequence 197-203 was predicted to contain the nuclear localization signal. There is no predicted trans-

membrane segment (using TMAP and PredictProtein), no mitochondrial targeting sequence (PSORT), and no secretory signal (SignalP).

[0127] Using PSI-BLAST on non-redundant database, amino acid sequence 1-42 of HCC-1 [Hcc-1] was matched to vertebrate heterogenous nuclear ribonucleoprotein with identities match of above 45%:

- Heterogenous nuclear ribonucleoprotein U (AF073992) of *Mus musculus* [Expect = 0.005, Identities = 21/42 (50%), Positives = 29/42 (69%)]
- SP120 (D14048) (nuclear scaffold protein that binds the matrix attachment region DNA) of *Rattus norvegicus* [Expect = 0.005, Identities = 21/42 (50%), Positives = 29/42 (69%)]
- ROU_HUMAN Heterogenous nuclear ribonucleoprotein U (HNRNP U) (Scaffold Attachment Factor A) (SAF-A) (Q00839) of *Homo sapiens* [Expect = 0.012 Identities = 20/42 (47%), Positives = 29/42 (68%)]
- hnRNP U protein (X65488) of *Homo sapiens* [Expect = 0.012, Identities = 20/42 (47%), Positives = 29/42 (68%)]
- Scaffold attachment factor A (AF068847) of *Xenopus laevis* [Expect = 0.021, Identities = 20/37 (54%), Positives = 26/37 (70%)]

[0128] Using FASTA3 on SWALL non-redundant database, [Hcc-1] HCC-1 was matched to various invertebrate translated proteins with E-value below 0.03:

- Q9VHC8 CG8149 protein of *Drosophila melanogaster* [Expect=8e-06]
- Q9N3G0 Hypothetical protein Y53G8AR.d of *Caenorhabditis elegans* [Expect=0.0005]
- Q9LZ08 Hypothetical 22.8 KDA protein of *Arabidopsis thaliana* [Expect=0.021]
- O74871 Conserved hypothetical protein of *Schizosaccharomyces pombe* (Fission yeast) [Expect=0.024]

[0128] Physically, this [Hcc-1] HCC-1 protein may have 2 to 3 domains from coiled-coil and low complexity region predictions:

- PredictProtein Coiled-Coil prediction – the coil is most probably at 30-51 positions. The next possible coiled-coil is at 146-160 positions. Coiled-coil most probably separates the different domains.
- COILS ver 2.2 (Lupas) – at aa 25 – 64 and aa 145 – 172.
- SEG Low Complexity regions predicted 2 regions: at aa 42-79 and aa 165-179.

***IN THE CLAIMS:***

Claims 3-14 and 18, have been canceled.

Claim 1 has been amended as follows:

1. (twice amended) An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 or a nucleotide sequence, having at least about 60% similarity to the full length of SEQ ID NO:1 or SEQ ID NO:3, that hybridizes to SEQ ID NO:1 or SEQ ID NO:3 under conditions of 0.1 x SSC buffer, 0.1% w/v SDS, at a temperature of at least 65°C, wherein an mRNA corresponding to said nucleic acid is differentially or preferentially expressed [said nucleic acid expresses an amount of mRNA that is differential or preferential] in human hepatocellular carcinoma tissue or tissue from pancreatic adenocarcinoma relative to other tissue in said subject and/or in subjects not diagnosed with this condition.